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Effects of Iontophoretically Applied Naloxone, Picrotoxin and Strychnine on Dorsal Horn Neuron Activities Treated with High Frequency Conditioning Stimulation in Cats

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Transcutaneous electrical nerve stimulation(TENS), acupuncture-needling, and electroacupuncture are useful non-ablative methods in medical practice for relief of pain. These procedures appear to work by causing an increased discharge in afferent nerve fibers which in turn modifies the transmission of impulses in pain pathways. It is known that the mechanism of analagesic effect via these maneuvers are variable depending on the stimulating parameters. For example, the endogenous opioid system is profoundly related to the mechanism when a peripheral nerve stimulation is applied with parameters of low frequency and high intensity. However, when stimulated with parameters of high frequency and high intensity, the reduced activity of dorsal horn neurons is only slightly reversed by a systemic administration of naloxone, a specific opiate antagonist. Thus, the present study was performed to investigate the neurotransmitter that concerns the mechanism of peripheral nerve stimulation with parameters of high frequency and high intensity. We used an iontophoretic application of antagonists of possible related neurotransmitters. The dorsal horn neuron activity which was evoked by squeezing the peripheral cutaneous receptive field, was recorded as an index of pain with a microelectrode at the lumbo-sacral spinal cord. Naloxone, picrotoxin and strychnine were applied at 200nA during a period of conditioning nerve stimulation. We observed the effects of these drugs on the change of dorsal horn neuron activities. The main results of the experiment can be summarized as follows. The spontaneous activity of dorsal horn neurons increased in the presence of glutamate and decreased with GABA. It did not change with naloxone, picrotoxin or strychnine. When naloxone was applied iontophoretically during peripheral nerve stimulation, there was no statistically significant analgesic effect compared with that of the control group. When picrotoxin was applied iontophoretically during peripheral nerve stimulation, the analgesic effect was reduced. When strychnine was applied, the analgesic effect was reduced but did not show a statistically significant difference with the control group. These results suggested that the GABAergic system may have been partially related in the analgesic action of peripheral nerve stimulation with parameters of high frequency and high intensity.

Key Words: Analgesia, peripheral nerve stimulation, iontophoresis, GABA, glycine, opiate

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One of the properties of pain sensation is that it is labile. Under some conditions, a given noxious stimulus may evoke a full pain reaction in an individual, whereas in other circumstances the same individual may fail to experience pain at all. Presumably this variability in the pain reaction is due in part to the operation of the endogenous control sys-

tem. It has been suggested that the variable nature of pain reaction casts on the presence of discrete nociceptive pathway called the gate control system(Melzack and Wall, 1965), another view is that there are specific nociceptive pathways whose activity is sometimes suppressed by the descending pain inhibitory system(Reynolds, 1969; Oliveras *et al.* 1974; Cannon *et al.* 1982 Fields and Basbaum, 1978), mainly via the endogenous opioid system (Hughes, 1975; Hughes *et al.* 1975; Goldstein, 1976; Simantov *et al.* 1976; Duggan *et al.* 1976; Guillemin *et al*, 1977; Basbaum and Fields, 1984).

Classically, transcutaneous electrical nerve stimulation(TENS) is a procedure of applying controlled, low-voltage electrical pulses to the nervous system by passing electricity through the skin via electrodes placed on the skin (Mannheinmer and Lampe, 1984). This therapy has found broad application because physicians and physical therapists have rapidly accepted the ease of application, efficacy, and lack of undesirable side effects. Acupuncture-needling in Oriental medicine and modified electroacupuncture are also used as non-ablative methods in medical practice for relief of acute and chronic pain. These procedures appear to work by stimulation of the peripheral nerve thereby cause an increased discharge in afferent nerve fibers which in turn modify the transmission of impulses in pain pathways (Chiang *et al.* 1973; Fleck, 1975; Levy and Matsumoto, 1975; Toda and Ichioka, 1978; Paik *et al.* 1982).

Meanwhile, it is known that the mechanisms of analgesic actions of these maneuvers are variable depending on the stimulating parameters. For example, in peripheral nerve stimulation(PNS) with low intensity and high frequency, the analgesic effect has a rapid onset, short duration, and segmental distribution, and it is not associated with the endogenous opioid system. This effect has been explained by the gate control theory(Melzack and Wall, 1965; Wagman and Price, 1969; Handwerker *et al.* 1975; Woolf and Wall, 1982; Chung *et al.* 1984a, 1984b). On the other hand, the analgesic effect produced by stimulation with high intensity and low frequency has a slow onset and long duration, and is profoundly related to the endogenous opioid system (Sjölund and Eriksson, 1979). However, when the PNS is applied with parameters of high frequency and high intensity, the activity of dorsal horn neurons is only slightly reversed by naloxone, a specific opiate antagonist, suggesting that other neurotransmitters such as GABA or glycine may be implicated in this mechanism. Also, the accumulation of K^+ ions in the narrow interstitial fluid in the spinal cord may lead to a reduction of excitability of the dorsal horn neurons(Nam *et al.* 1991).

The present study was performed to investigate the neurotransmitter that concerns the mechanism of PNS with parameters of high frequency and high intensity by using iontophoretic applications of some antagonists of the neurotransmitters.

MATERIALS AND METHODS

Experiments were performed on a total of 26 adult cats of either sex weighing 2.0 ~ 3.0 kg. Cats were anesthetized initially with a intramuscular injection of ketamine hydrochloride (25 mg/kg). Under initial anesthesia, both the trachea and the external jugular veins were cannulated and each animal was paralyzed with gallamine triethiodide (Flaxedil, an injection of 20 mg i.v. followed by infusion at 4 mg/kg/hr). End-tidal CO_2 concentration was monitored and maintained at 3.5~4.5% throughout the experiment.

Rectal temperature was kept near 37℃ using a heating blanket.

Decerebration was done by ligations of the basilar artery and bilateral common carotid arteries. Laminectomies were made at spinal levels L1 to S2. For test stimulation, both common peroneal nerve and tibial nerve were isolated from the surrounding connective tissue, and placed on a pair of platinum bipolar electrodes.

Each animal was fixed to a stereotaxic unit, and mineral oil pools were made around exposed spinal cord and peripheral nerves to prevent drying. The temperature of the pool

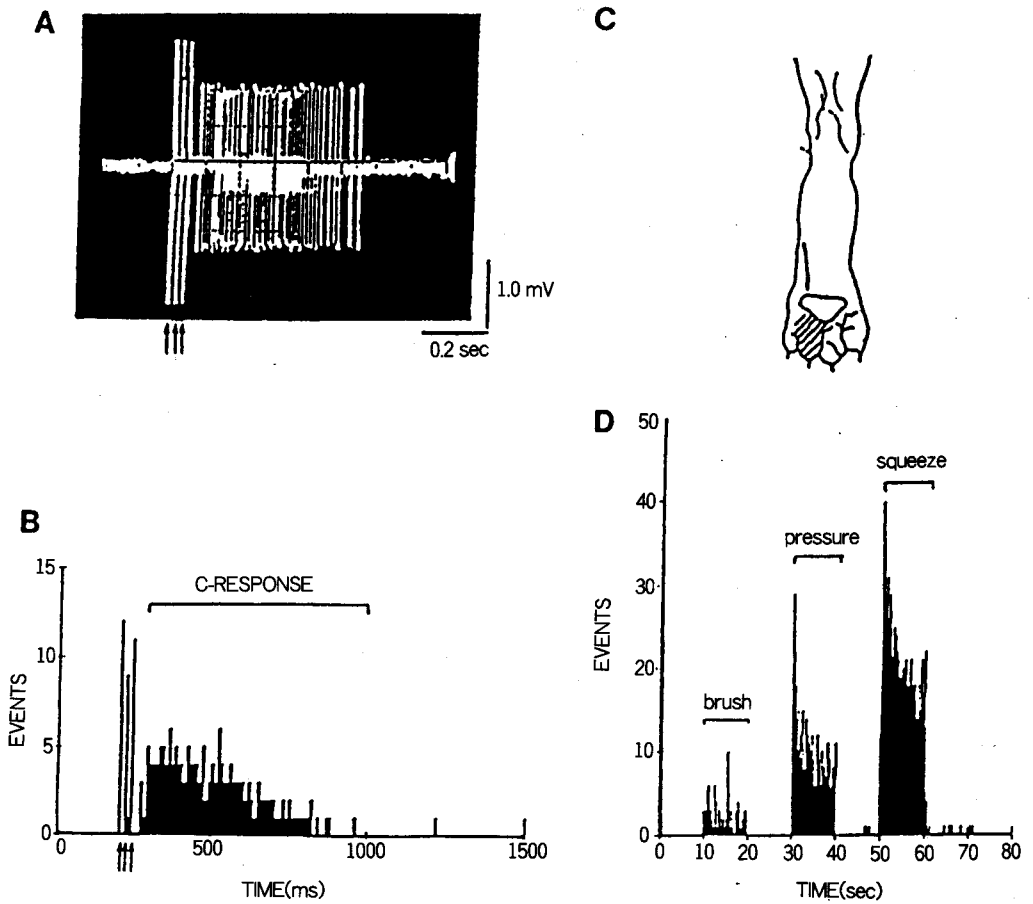

Fig. 1. *An example of the recording of activities of a dorsal horn neuron. A: three consecutive electrical pulses (10mA, 500 μsec duration, an intensity suprathreshold for C fibers) were applied to the common peroneal nerve at the times indicated by the arrows. Spikes were photographed on the oscilloscope face. B: the post-stimulus time histogram shows A and C-fiber evoked response by the test stimuli as in A. The histogram was complied from responses to 3 successive stimuli. Bin widths were 10msec. C: the receptive field of the neuron is indicated by the hatched area. D: the neuron responded to both innocuous and noninnocuous mechanical stimuli within the receptive field, representing a wide dynamic range cell. Bin widths were 200msec.*

was maintained by heating and immersing coils into the pool.

For recording of dorsal horn neuron activities and injection of drugs, seven barreled electrodes were inserted using a pulse motor microdrive manipulator into the lumbosacral spinal cord where the largest cord dorsum potential could be recorded upon stimulation of the common peroneal nerve. The central barrel was filled with 2M NaCl solution(tip resistance ; 1.8~15Ω) for recording. Other barrels were filled with sodium-L-glutamate(GLU, 0.2M, pH 8.0), γ-aminobutyric acid(GABA, 0.2M, pH 3.5), naloxone hydrochloride(NAL, 0.1M, pH 4.5), picrotoxin(PCT, 0.01M in 165mM NaCl solution, pH 5), strychnine hydrochloride(STR, 0.1M, pH 6.0) and were used for drug injection. A barrel was filled with NaCl(165mM) and used for current compensation. A 5nA retaining current was continuously injected to mini-

mize the diffusional leak of drugs.

When a single unit activity was obtained, recording of dorsal horn neuron activity elicited by electrical or natural stimuli was started. Activities of wide dynamic range(WDR) or high threshold(HT) cells elicited by squeezing each receptive field were used as an index of pain(Fig. 1).

As conditioning stimulus, square-wave electrical pulses were applied to ipsilateral tibial nerve at 50Hz for 30 seconds. Strength of the conditioning stimulus was adjusted to activate all fiber groups, including C-fibers (10mA intensity, 500 μsec duration). The dorsal horn neuron activities were recorded before and at 10, 60 and 110 seconds after peripheral nerve stimulation for a period of 10 seconds. The stimulation effects were compared both in the presence of drug and not. Results are expressed as the meas±S.E. The student t-tests (paired) were used for statistical analysis of the results. Two tailed p values less than 0.05 were considered significant.

RESULTS

Effects of iontophoretically applied drugs on the spontaneous activity of dorsal horn neurons

Iontophoretic application of NaCl had no effect on spontaneous activity, but GLU produced increased activity(Fig. 2. a) in a dose dependent manner(Fig. 2. c). However GABA decreased spontaneous activity(Fig. 2. a).

Other drugs, NAL, STR, and PCT, had no effect on the spontaneous activity. Thus there would be little or no tonic effects elicited by opioid, glycine or GABA on dorsal horn neurons.

Effects of naloxone on the activity of dorsal horn neurons treated with high frequency conditioning stimulation

Naloxone, a well-known opioid antagonist, was applied during the period of conditioning stimulus. In the control state, evoked reponses by squeeze were reduced significantly($p<0.05$) to 6.70±2.42%, 32.16±6.65%, and 55.60±8.49% (Mean±S.E.) of pre-stimulus value at 10, 60, and 110 seconds after conditioning stimulation, respectively. Under naloxone, these were also reduced significantly($p<0.05$) to 8.66±3.24%, 24.41±3.95%, and 48.98±7.89% of the pre-stimulus value at 10, 60, and 110 seconds after conditioning stimulation, respectively. And naloxone did not show any significant difference between control and naloxone state(Fig. 3).

Effects of picrotoxin on the activity of dorsal horn neurons treated with high frequency conditioning stimulation

Picrotoxin, a GABA antagonist, was applied during the period of conditioning stimulus. In the control state, evoked reponses by squeeze were reduced significantly($p<0.05$) to 4.29±1.15 %, 30.56±8.05%, and 55.30±10.10%(Mean±S.E.) of pre-stimulus value at 10, 60, and 110 seconds after conditioning stimulation, respectively. Under picrotoxin, they also reduced significantly($p<0.05$) to 15.93±5.70%, 38.89±7.37, and 70.00±11.39% of the pre-stimulus value at 10, 60, and 110 seconds after conditioning stimulation, respectively. And picrotoxin showed a significant difference between control and picrotoxin state at 10 seconds after conditioning stimulation(Fig. 4).

Effects of strychnine on the activity of dorsal horn neurons treated with high frequency conditioning stimulation

Strychnine, a glycine antagonist, was applied during the period of conditioning stimulus. In the control state, evoked reponses by squeeze were reduced significantly($p<0.05$) to 2.88±0.84 %, 26.87±8.41%, and 46.24±9.53%(Mean±S.E.) of pre-stimulus value at 10, 60, and 110 seconds after conditioning stimulation, respectively. Under the influence of strychnine, they also reduced significantly($p<0.05$) to 10.13±4.83 %, 40.80±11.10%, and 75.00±20.30% of the pre-stimulus value at 10, 60, and 110 seconds after conditioning stimulation, respectively. Moreover, the strychnine did not show any significant difference between the control and the strychnine state(Fig. 5).

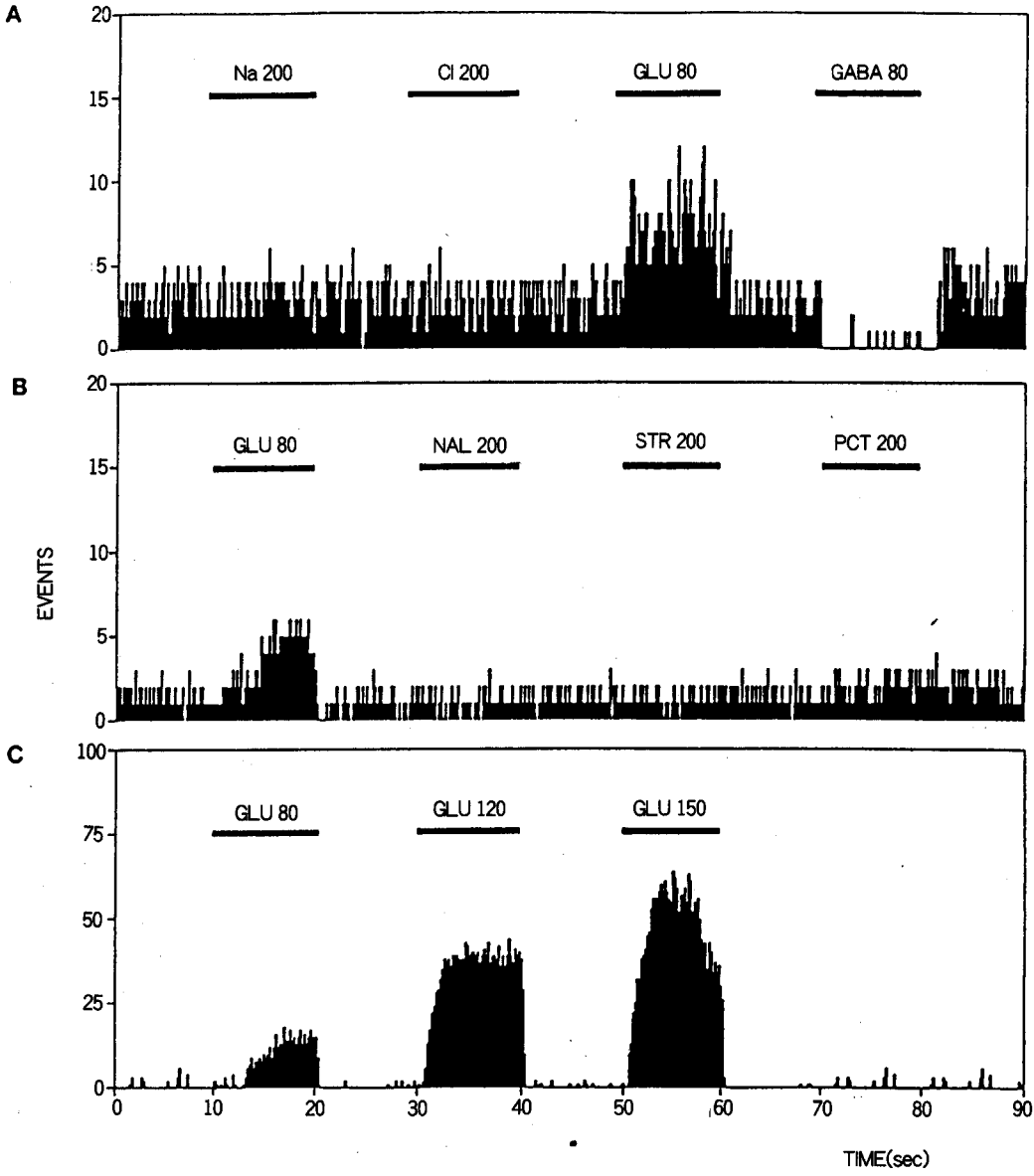

Fig. 2. *Effects of drugs on spontaneous activity of dorsal horn neurons. The bars refer to those periods during which the drugs were injected and the numbers refer to the amounts of injected drugs in the currents(nA). A: A peristimulus histogram shows that the glutamate increases and GABA decreases the activity of the neuron but the neuron dose not response to injected iontophoretic current. Bin widths were 200msec. B: Naloxone, strychnine and picrotoxin did not influence the spontaneous activity. C: The neuron responded to glutamate in a dose dependent manner.*

Na: sodium ion, Cl: chloride ion, GLU: glutamate, GABA: γ-aminobutyric acid, NAL: naloxone, STR: strychnine, PCT: picrotoxin

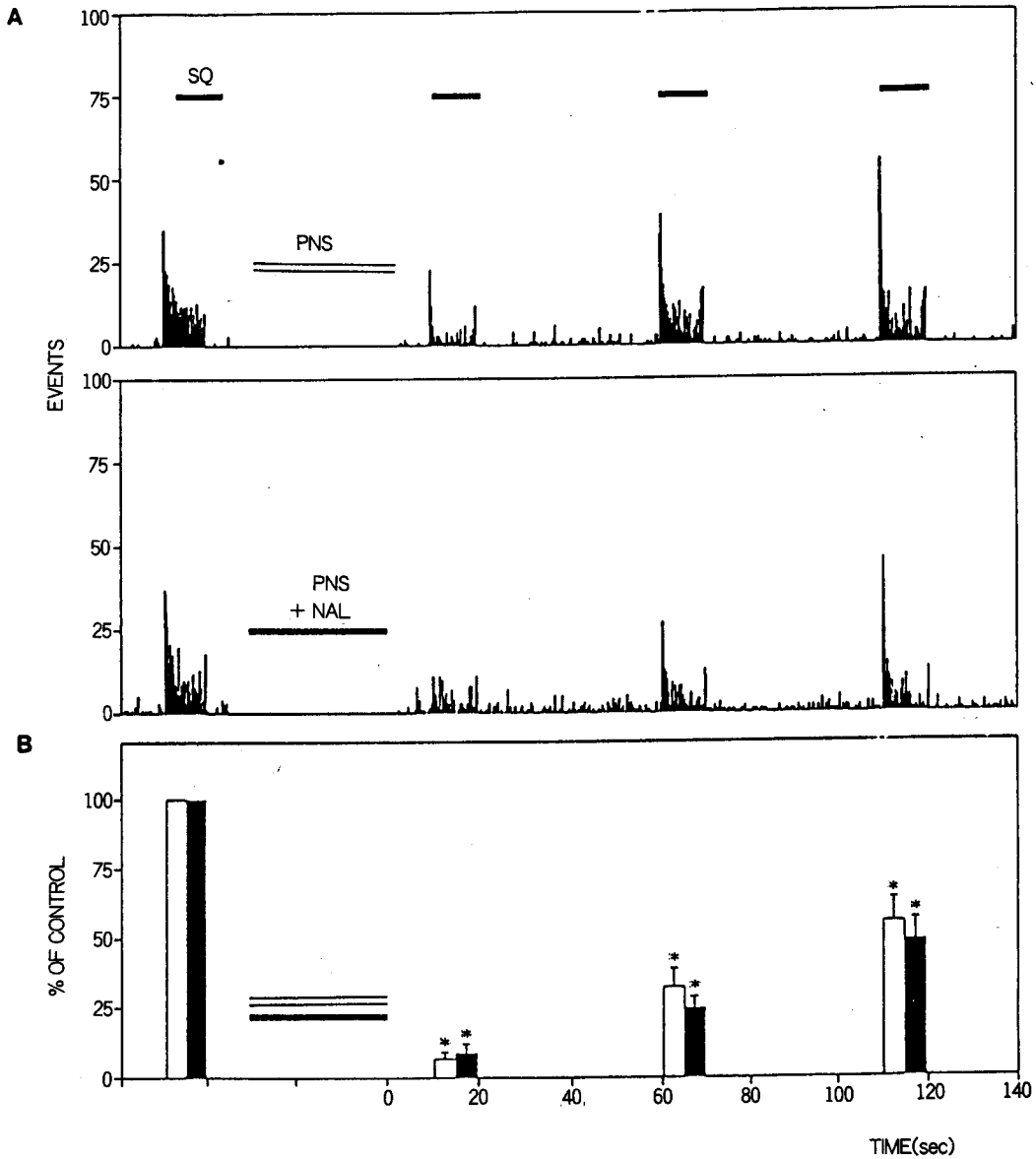

Fig. 3. *Effects of naloxone on dorsal horn neuron activity when treated with a high frequency conditioning stimulation. A: The effect of peripheral nerve stimulation on the evoked activity by squeezing the receptive field was compared between states without(upper panel) and with(lower panel) naloxone. Bin widths were 200msec. The bars refer to those periods during which the indicated treatments were performed. B: Activities are expressed as a percentage of the pre-stimulus control values.*

**: $p<0.05$ (compare with pre-PNS value)*

SQ: squeeze, PNS: peripheral nerve stimulation, NAL: naloxone

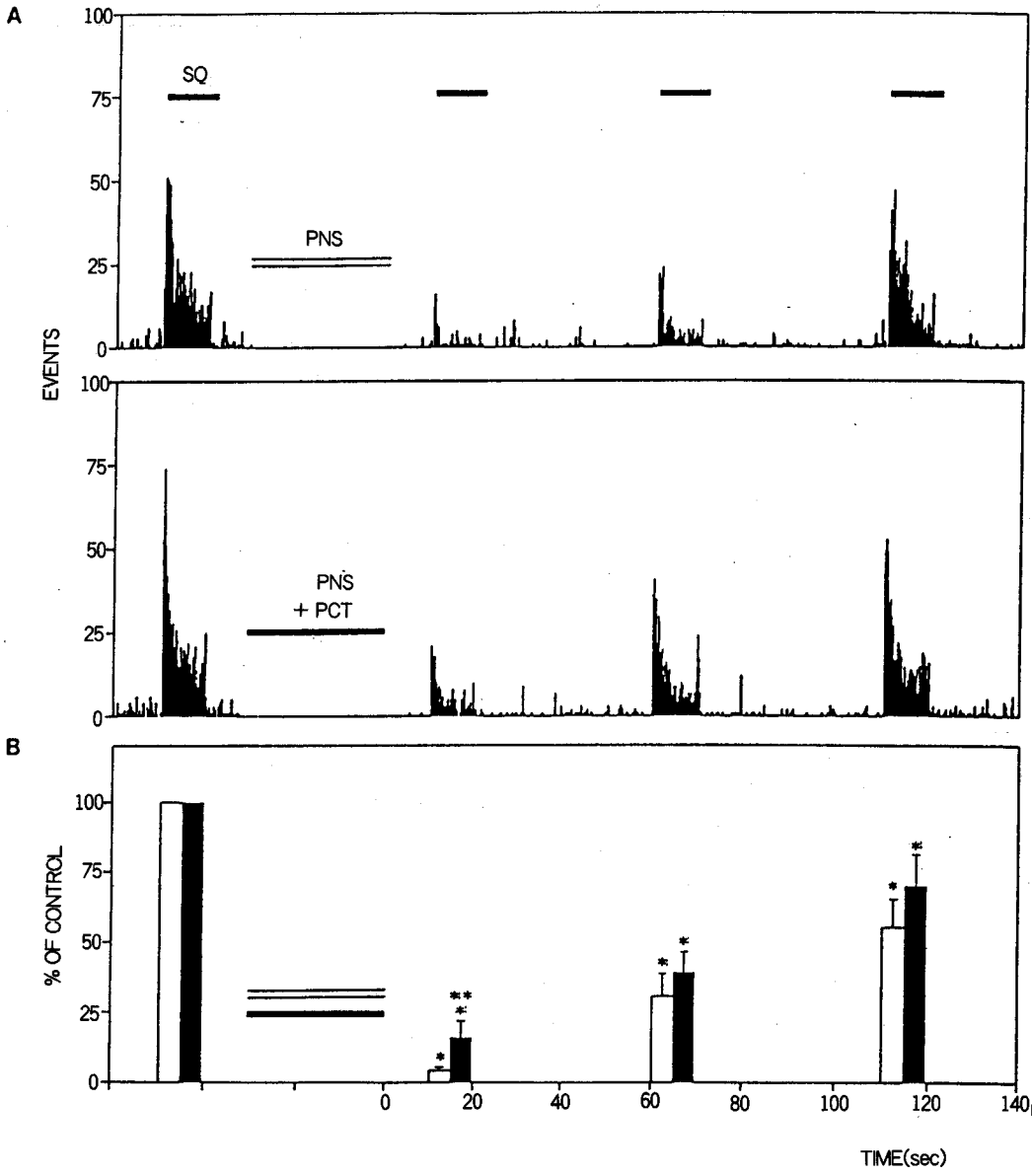

Fig. 4. *Effects of picrotoxin on dorsal horn neuron activity when treated with a high frequency conditioning stimulation. A: The effect of peripheral nerve stimulation on the evoked activity by squeezing the receptive field was compared between states without(upper panel) and with(lower panel) picrotoxin. Bin widths were 200msec. The bars refer to those periods during which the indicated treatments were performed. B: Activities are expressed as a percentage of the pre-stimulus control values.*

**: $p<0.05$ (compare with pre-PNS value)*

***: $p<0.05$ (compare with control group)*

SQ: squeeze, PNS: peripheral nerve stimulation, PCT: picrotoxin

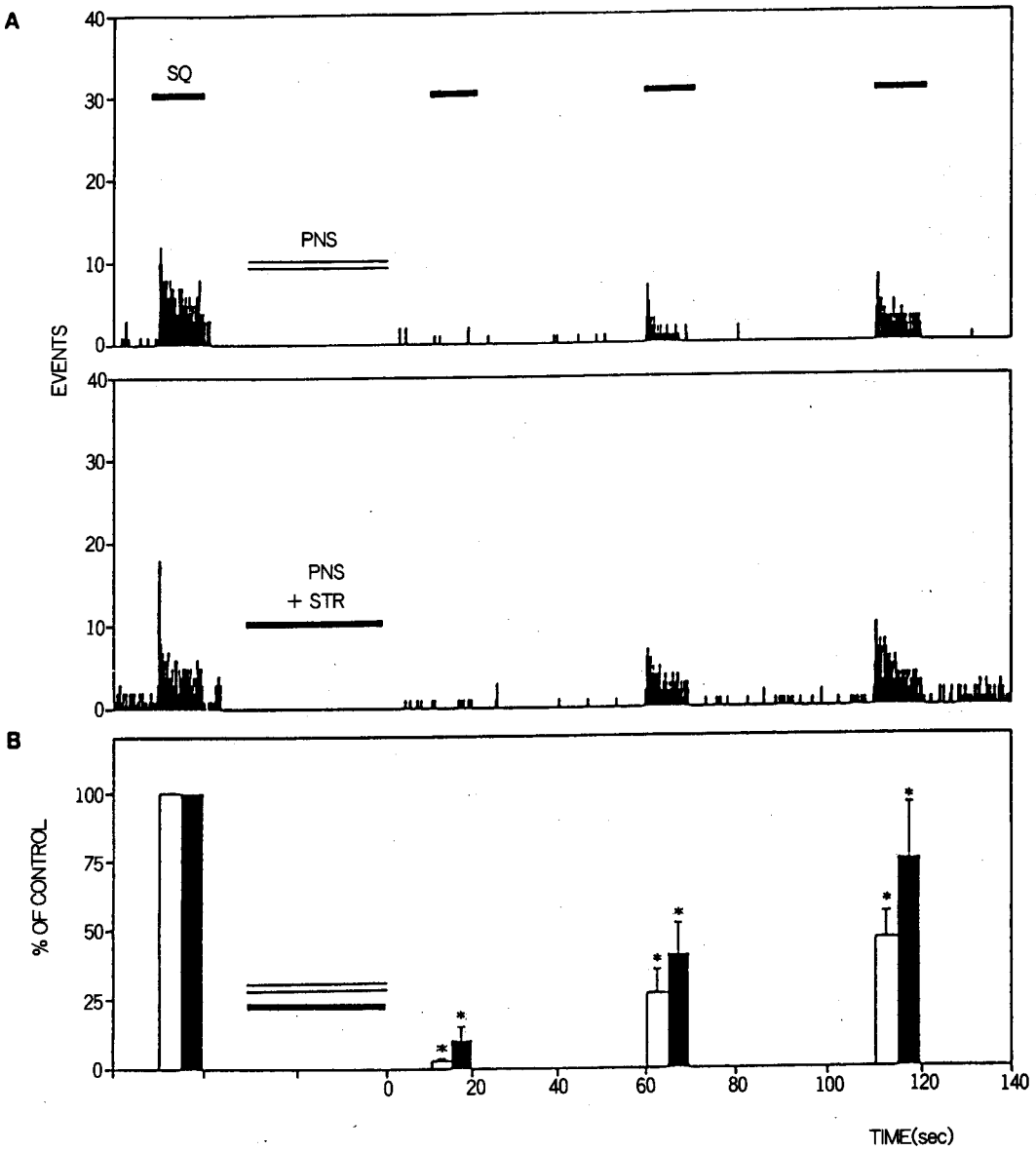

Fig. 5. *Effects of strychnine on dorsal horn neuron activity when treated with a high frequency conditioning stimulation. A: The effect of peripheral nerve stimulation on the evoked activity by squeezing the receptive field was compared between states without(upper panel) and with(lower panel) strychnine. Bin widths were 200msec. The bars refer to those periods during which the indicated treatments were performed. B: Activities are expressed as a percentage of the pre-stimulus control values.*
**: $p<0.05$ (compare with pre-PNS value)*
SQ: squeeze, PNS: peripheral nerve stimulation, STR: strychnine

DISCUSSION

Pain is an unpleasant sensation that occurs in response to tissue injury, producing arousal and motivation to escape the offending stimulus. Pain and its related phenomena continue to be an important problem in medicine.

One of the therapeutic methods for reducing pain is to exploit the patient's own in-built pain control mechanisms; for example peripheral nerve stimulation. The peripheral nerve stimulation is applied either transcutaneously (TENS) or via implanted electrodes and using various stimulation parameters. Many studies have been reported the development of models or parameters for better effects(Campbell, 1981; Paik *et al.* 1985).

The mammalian central nervous system possesses a well-defined network or system that modulates nociceptive transmission. In 1965, Melzack and Wall suggested the gate control theory that nonpainful stimulation in the PNS can interfere with the relay of the sensation of pain to higher centers. Thus, this theory may explain in part why PNS may diminish or abolish the sensation of pain. And there is a great deal of evidence of the presence of an endogenous analgesic system mediated by a morphine-like substance. In addition there exist discrete brainstem sites such as periaqueductal grey or nucleus raphe magnus which when electrically or chemically stimulated, are capable of suppressing pain transmission(Reynolds, 1969; Young, 1989). In addition to this, there are neurons containing enkephaline or dynophin in substantia gelatinosa(Aronin *et al.* 1981), and in spinal animals the analgesic effect is thought to be induced by this spinal endogenous opioid system(Woolf *et al.* 1980; Yaksh and Elde, 1981).

The analgesic effects of PNS are well known, but the characteristics and the mechanism of the analgesic effects are diverse according to the stimulating parameters(Cheng and Pomeranz, 1979; Salar *et al.* 1981; Chung *et al.* 1984c; Nam *et al.* 1991). In PNS with low intensity and high frequency the analgesic effect has rapid onset, short duration, and segmental distribution, and it is not associated with the endogenous opioid system. This analgesic effect is explained in the gate control theory(Melzack and Wall, 1965; Handwerker *et al.* 1975; Woolf and Wall, 1982; Chung *et al.* 1984a, 1984b). However, the analgesic effect produced by stimulation with high intensity and low frequency is of slow onset and long duration, and is profoundly related to the endogenous opioid system(Sjölund and Eriksson, 1979). Stimulation with high frequency and high intensity also can induce an analgesic effect, but the effects were partially reversed by systemic naloxone administration, with no difference between decerebrated and spinalized animals(Nam *et al.* 1991).

In this experiment the iontophoretical application of naloxone showed either no or little effect. To elucidate the possibility of intervening of inhibitory neurotransmitters, we used picrotoxin, a GABA antagonist and strychnine, a glycine antagonist. Strychnine showed no influence on the reduced activity of the spinal dorsal horn neurons. In the case of picrotoxin, it showed a little but significant reversed effects on the analgesic effect of peripheral nerve stimulation.

There are many reports that GABA plays a role in the pain system. GABA mediated inhibitory circuits in the spinal cord have already been proven and evidences of GABA-immunoreactive terminals synapse on primate spinothalamic tract cell are reported(Liu *et al.* 1992; Carton *et al.* 1992; Powell and Todd, 1992). Administration of GABA-mimetic drug could produce analgesic effects(Aley and Kulkarni, 1989; Otsuka and Yanagisawa, 1990; Edwards *et al.* 1990). The effect of GABA is thought to work in a phasic manner, not in a tonic manner. In the results of this experiment, picrotoxin showed no effects on spontaneous activity of dorsal horn neurons. In an *in vivo* microdialysis study of GABA in rats, GABA release was increased by electrical spinal cord stimulation(Linderoth *et al.* 1994).

Picrotoxin acts on the $GABA_A$ receptors. There is a lot of evidence that $GABA_B$ receptors also participate in the pain system. Baclofen, a $GABA_B$ antagonist, has the effect

of relieving pain(Ochs, 1994; Aley and Kulkarni, 1991). There is also evidence that the GABAergic system acts on chronic pain. In a chronic pain model, intrathecal strychnine or bicuculline administration enhances thermal hyperalgesia(Yamamoto and Yaksh, 1993).

Brief high frequency electrical stimulation of spinal afferent can induce a long-term potentiation(LTP) or long-term depression(LTD) in the spinal dorsal horn neuron(Randic *et al.* 1993). In spinal cord slice preparations, tetanic stimulation with parameters similar with that in our experiment (high frequency trains of 100Hz) induced LTP or LTD. As in the hippocampus, primary afferent fibers use glutamate as their transmitter in spinal cord. However, in the mechanisms of LTD the involvement of the NMDA receptor was excluded and the non-NMDA receptors played a role(Randic *et al.* 1993). These results demonstrate that distinct and long lasting modulation in synaptic efficiency can be induced at primary afferent synapses with spinal dorsal horn neuron by high-frequency stimulation of dorsal root afferent and that these changes may be physiologically relevant for transmission and integration of sensory information, including pain. In our experiment the depression lasted a few minutes on average and thus the depression would be short term rather than long term, but in some cells the depression lasted for a long period. Thus the LTD mechanism may play a role as part of the mechanism.

Another mechanism that might contribute to the reduced activity of dorsal horn neuron is desensitization of the postsynaptic receptors (Linden *et al.* 1991). As for the presynaptic mechanism, the reduced activity may be due to a decrease in transmitter release. There are at least two presynaptic mechanisms that imight decrease the release of excitatory transmitter from the primary afferents: first, the activation of a presynaptic inhibitory pathway(Nicoll and Alger, 1979; Nicoll *et al.* 1990), and second the reduced efficacy of the transmitter release machinery of the synapse following high-frequency stimulation, as in depletion of transmitters. The involvement of glycine-mediated presynaptic inhibition was ruled out when we found that the blockade of the glycine receptors by an antagonist strychnine did not affect the peripheral nerve stimulation. Moreover, GABA-mediate inhibition might act. Beside this, it is possible that high-frequency stimulation produces excitotoxic damage in the postsynaptic neurons or some generalized loss of postsynaptic excitability or damage or fatigue to the stimulated inputs. Kritz(1975) demonstrated that when the peripheral nerve is stimulated at high frequency, K^+ accumulates in the narrow extracellular space. Thus the increased K^+ concentration makes the excitability of the dorsal horn neuron low.

This suggests changes in the passive properties of the dorsal horn neurons due to activation of postsynaptic inhibitory pathway by PNS. Other neurotransmitter or modulator maybe concern. Presynaptic disorder, postsynaptic receptor desensitization or nonspecific reduction in excitability of dorsal horn neurons may also play a role. Our results suggested that the GABAergic system is in part implicated in the mechanism of analgesic effect of peripheral nerve stimulation with high frequency and high intensity.

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